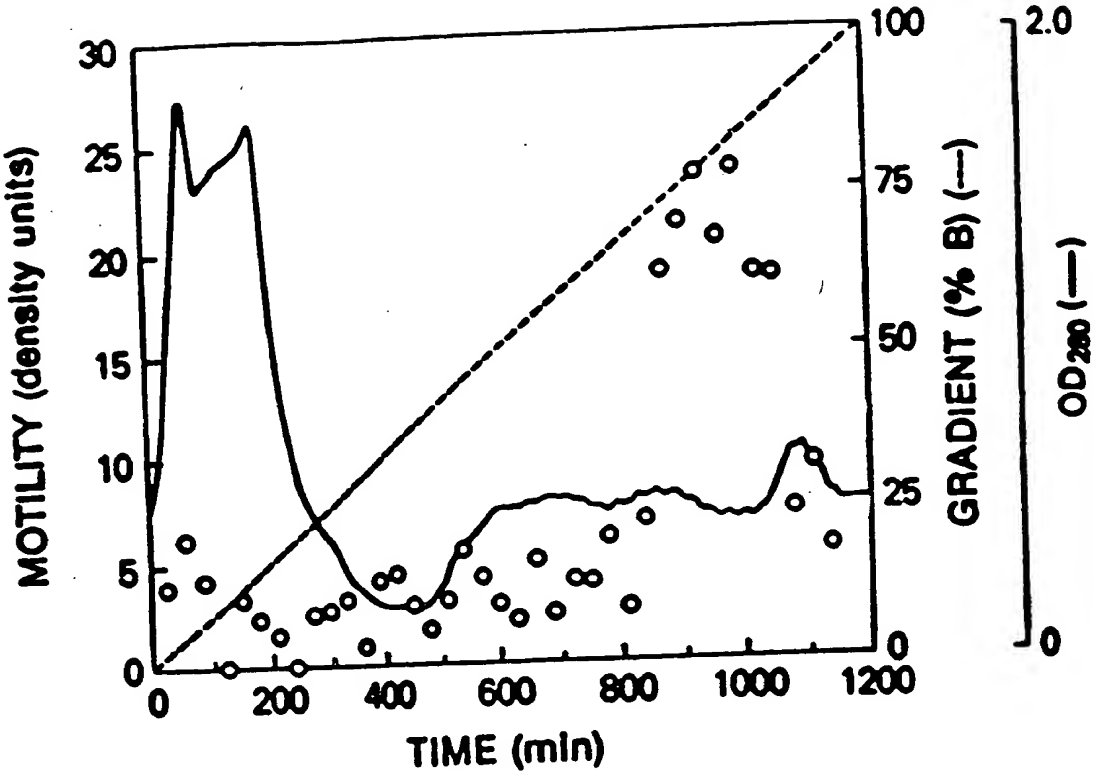

MOTILITY (density units)
30
25
20
15
10
5
0
0
200
400
600
800
1000
1200
TIME (min)
100
75
50
25
0
GRADIENT (% B) (—)
2.0
0
OD280 (—)

FIGURE 1

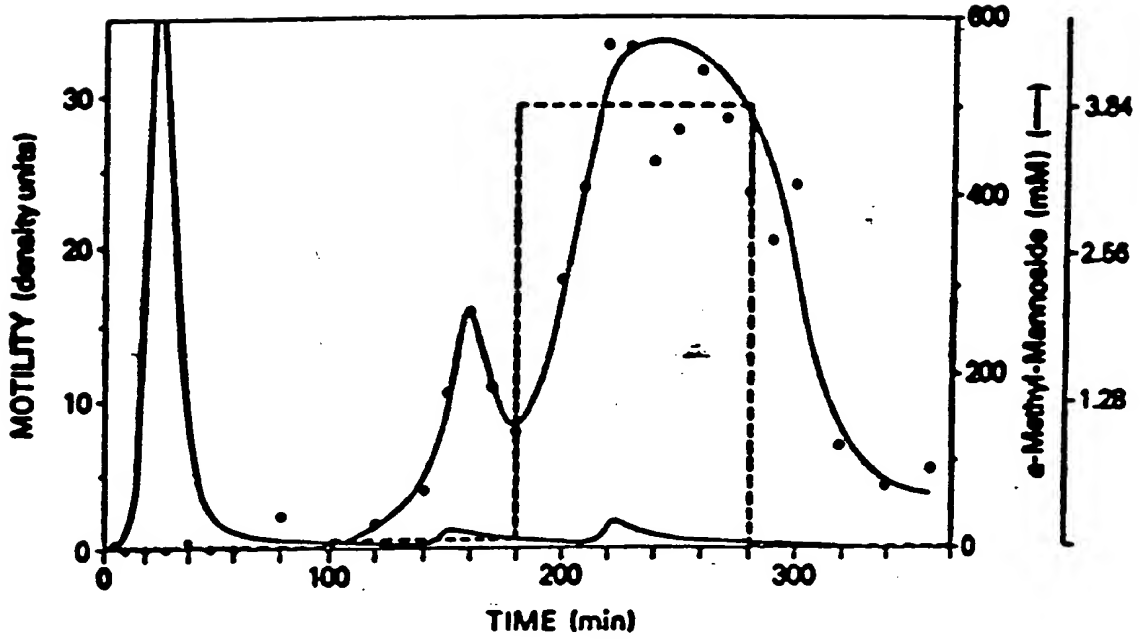

MOTILITY (density units)
30
20
10
0
0
100
200
300
TIME (min)
600
400
200
0
α-Methyl-Mannoside (mM)
3.84
2.56
1.28

FIGURE 2

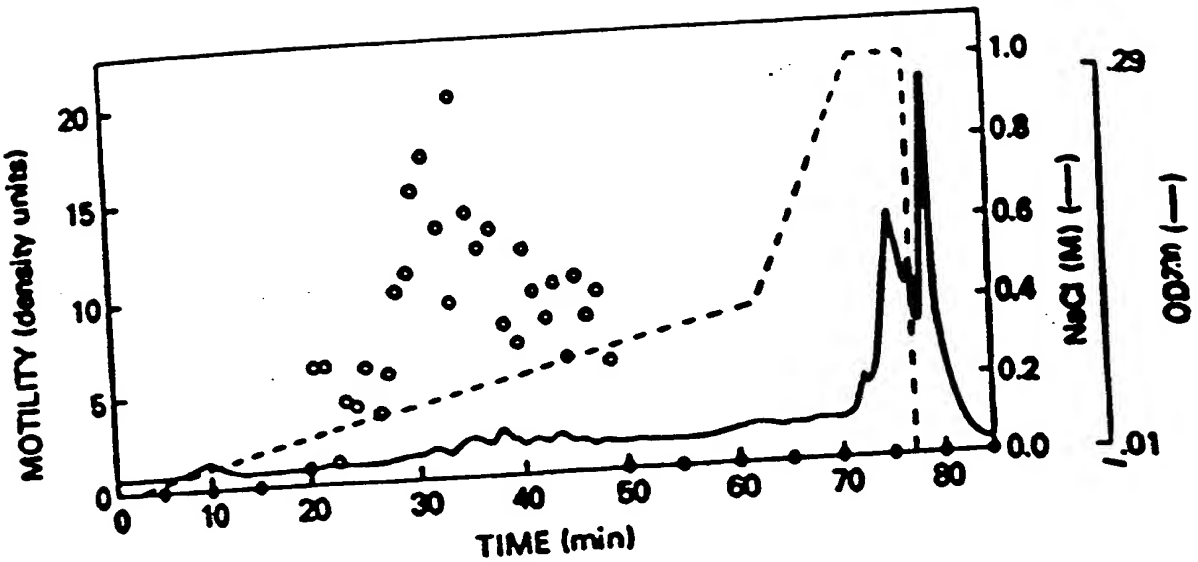

MOTILITY (density units)
20
15
10
5
0
0
10
20
30
40
50
60
70
80
TIME (min)
1.0
0.8
0.6
0.4
0.2
0.0
NaCl (M) (—)
.29
.01
OD570 (—)

FIGURE 3

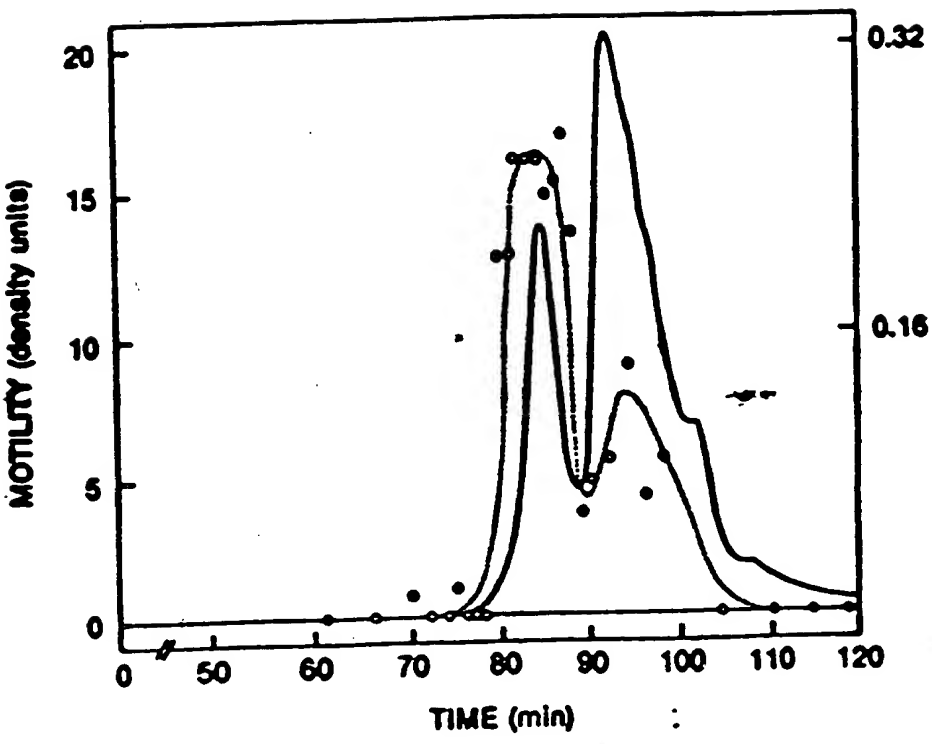


FIGURE 4

EI004875217US

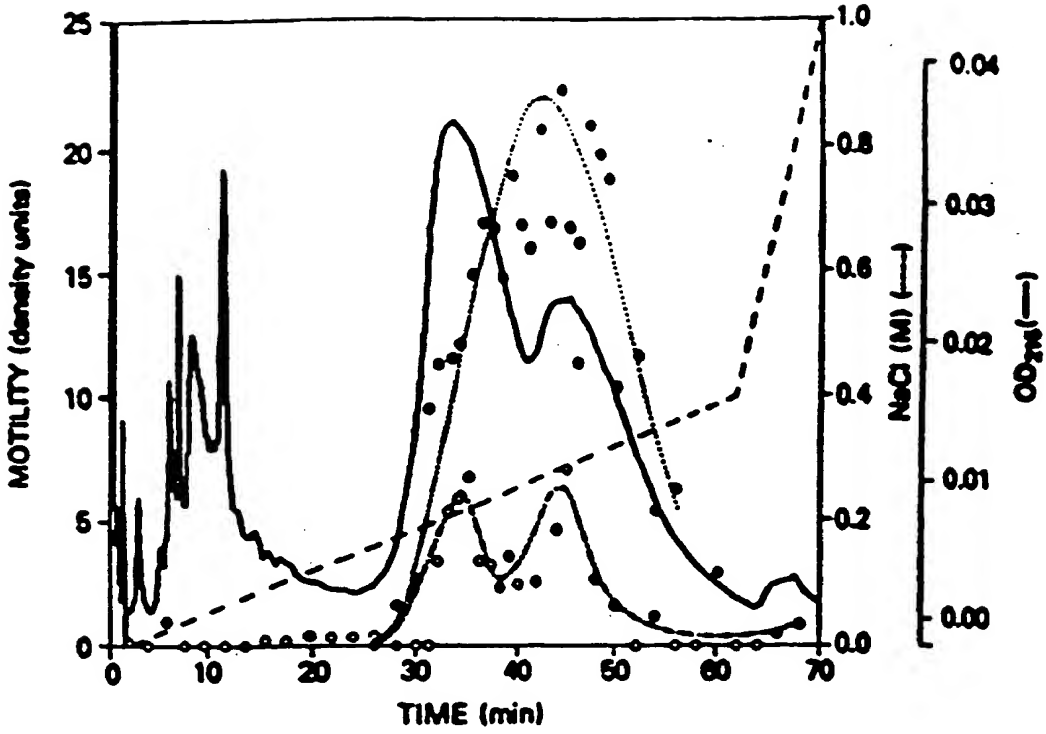

MOTILITY (density units)
TIME (min)
NaCl (M) (—)
OD_{750} (—)
0
5
10
15
20
25
0
10
20
30
40
50
60
70
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.01
0.02
0.03
0.04

FIGURE 5

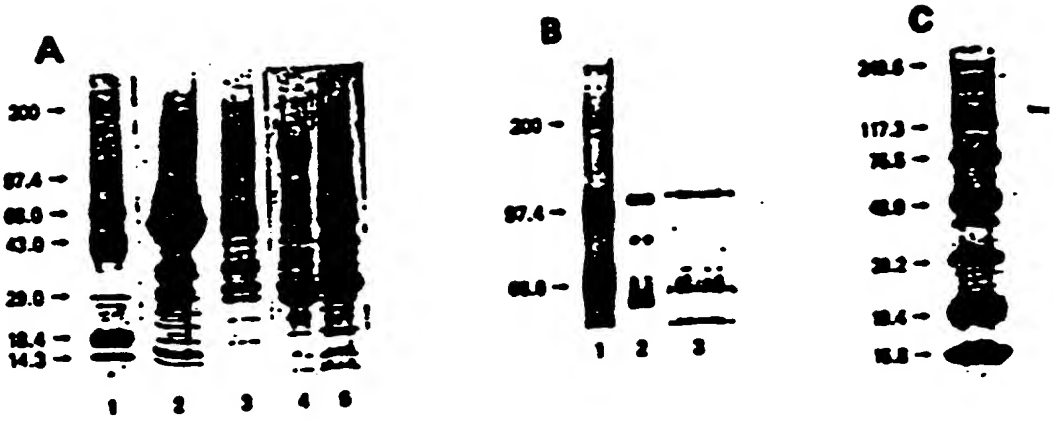

A
200
97.4
68.0
43.0
29.0
18.4
14.3
1 2 3 4 5
B
200
97.4
68.0
1 2 3
C
200.0
117.3
78.5
49.0
29.2
18.4
14.3
1

FIGURE 6

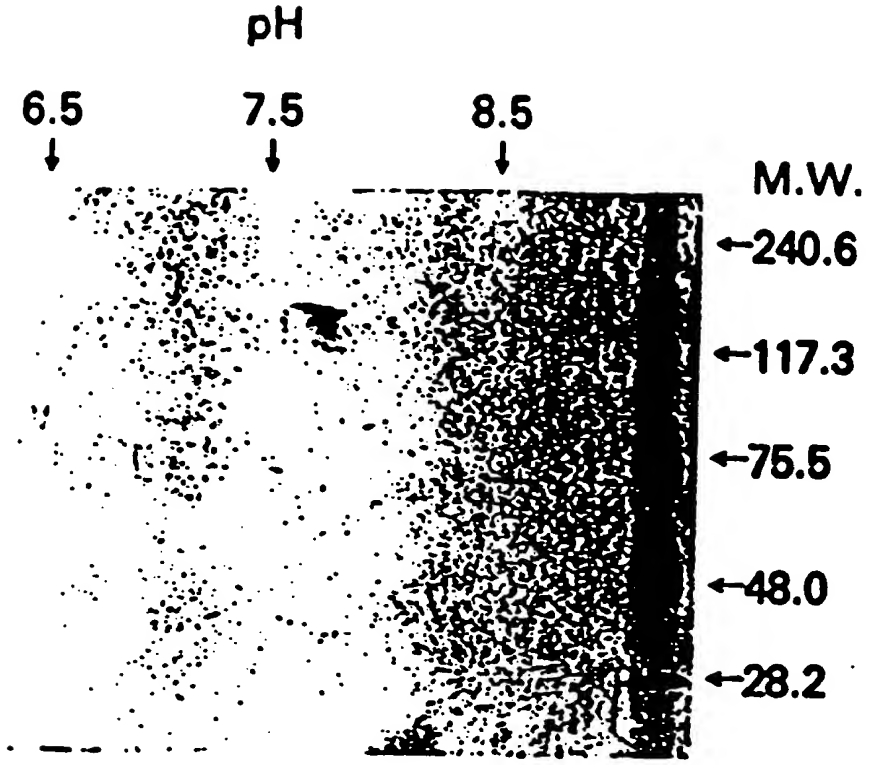


FIGURE 7

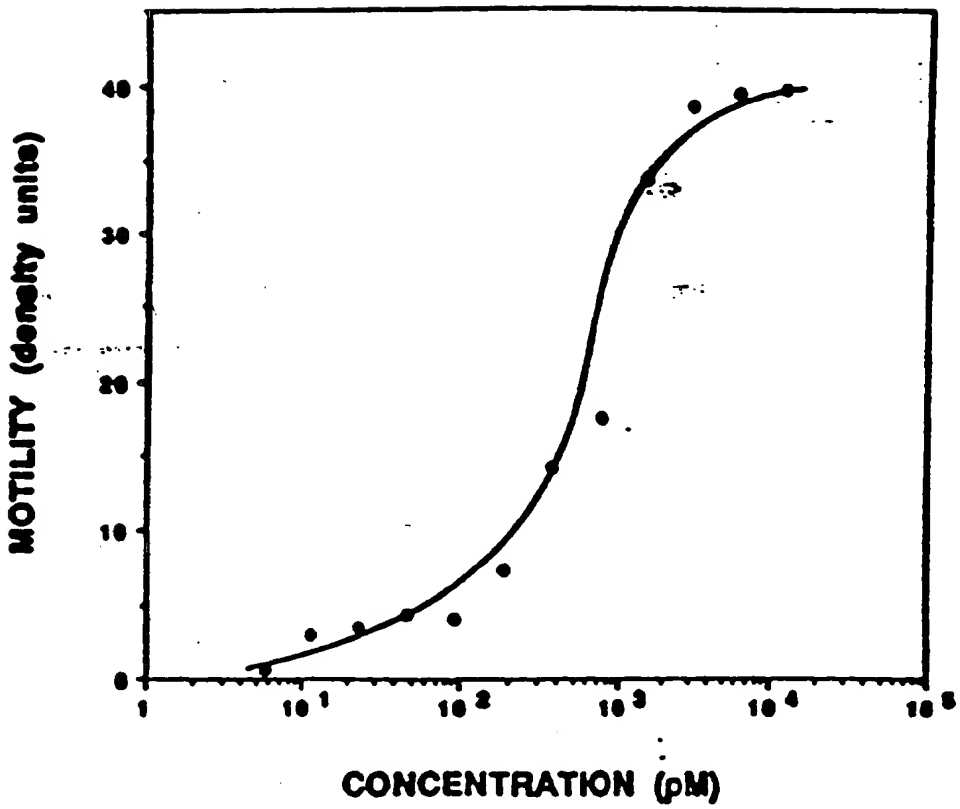

MOTILITY (density units)
40
30
20
10
0
1
10^1
10^2
10^3
10^4
10^5
CONCENTRATION (pM)

FIGURE 8

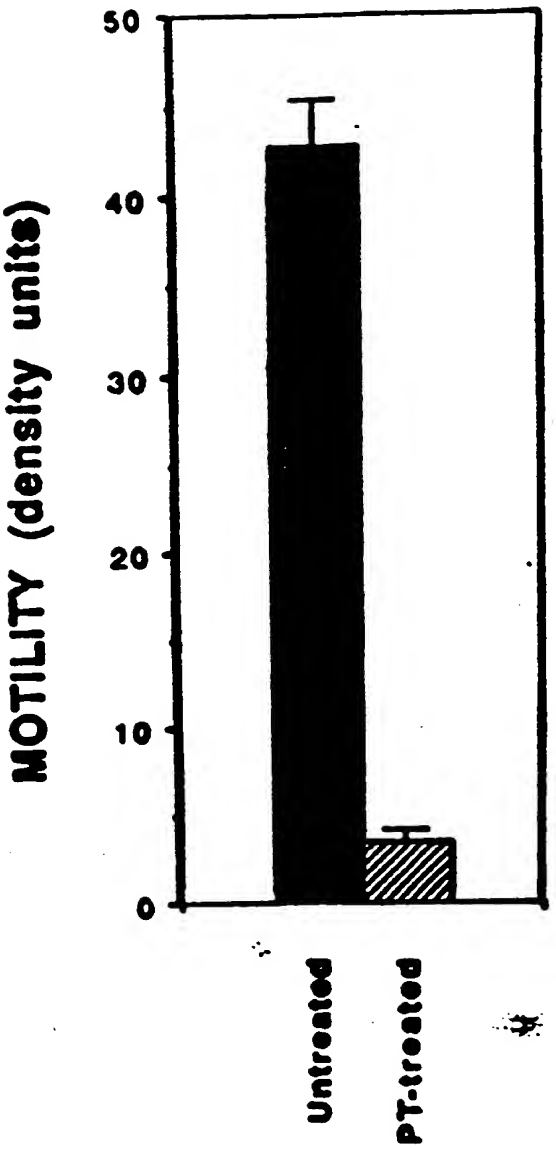


FIGURE 9

FIG. 10

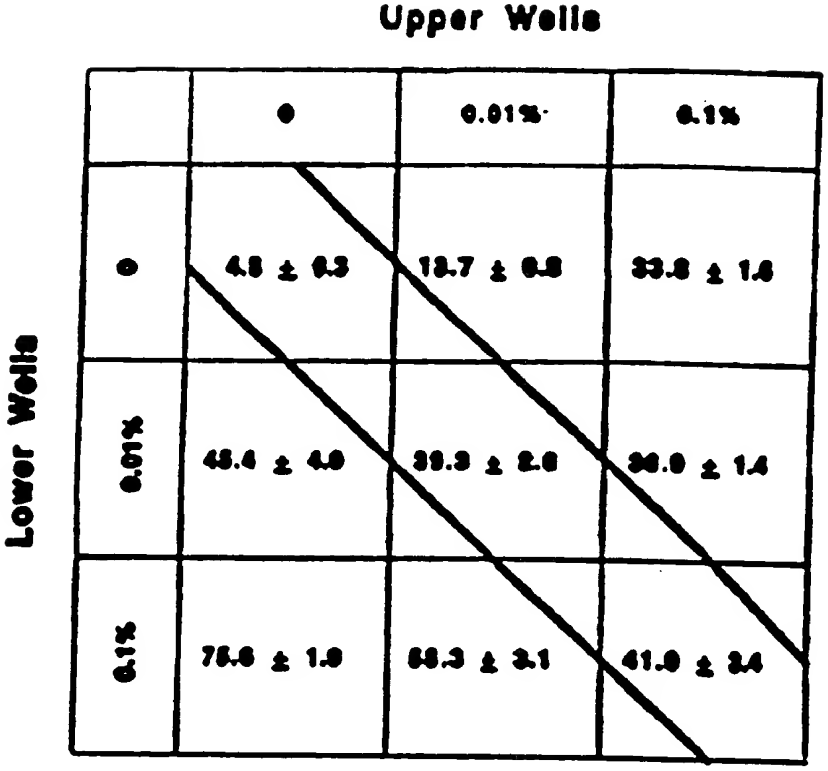
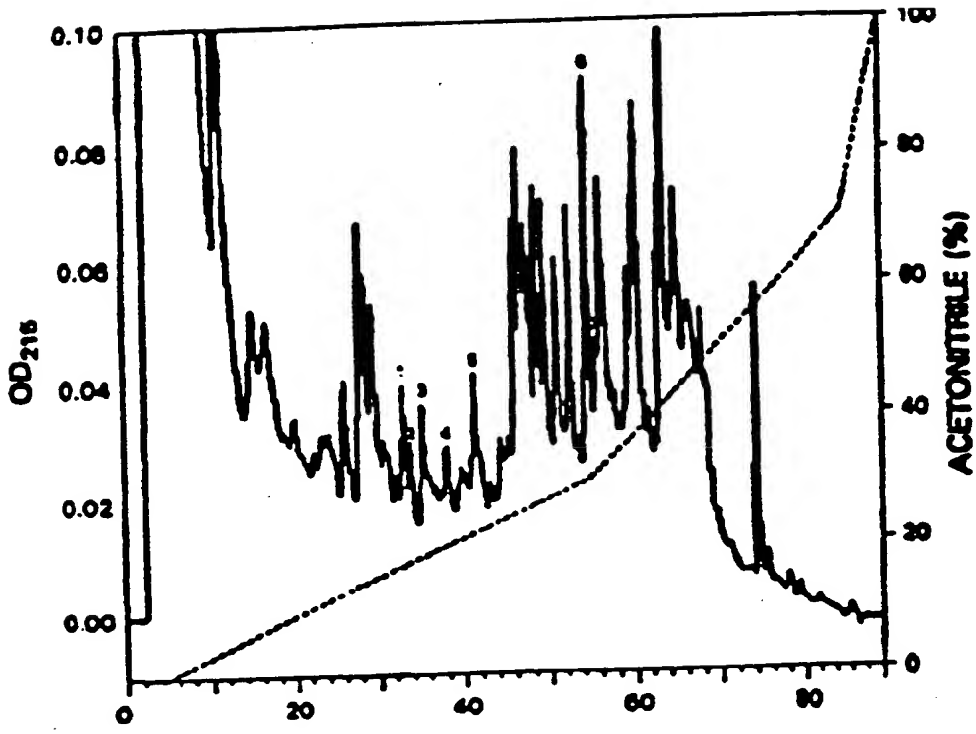

Upper Wells
Lower Wells
0
0.01%
0.1%
0
0.01%
0.1%
4.8 ± 0.3
18.7 ± 0.8
33.8 ± 1.6
48.4 ± 4.9
39.3 ± 2.6
36.8 ± 1.4
76.6 ± 1.9
58.3 ± 3.1
41.8 ± 3.4

FIG. 11



TIME (min)

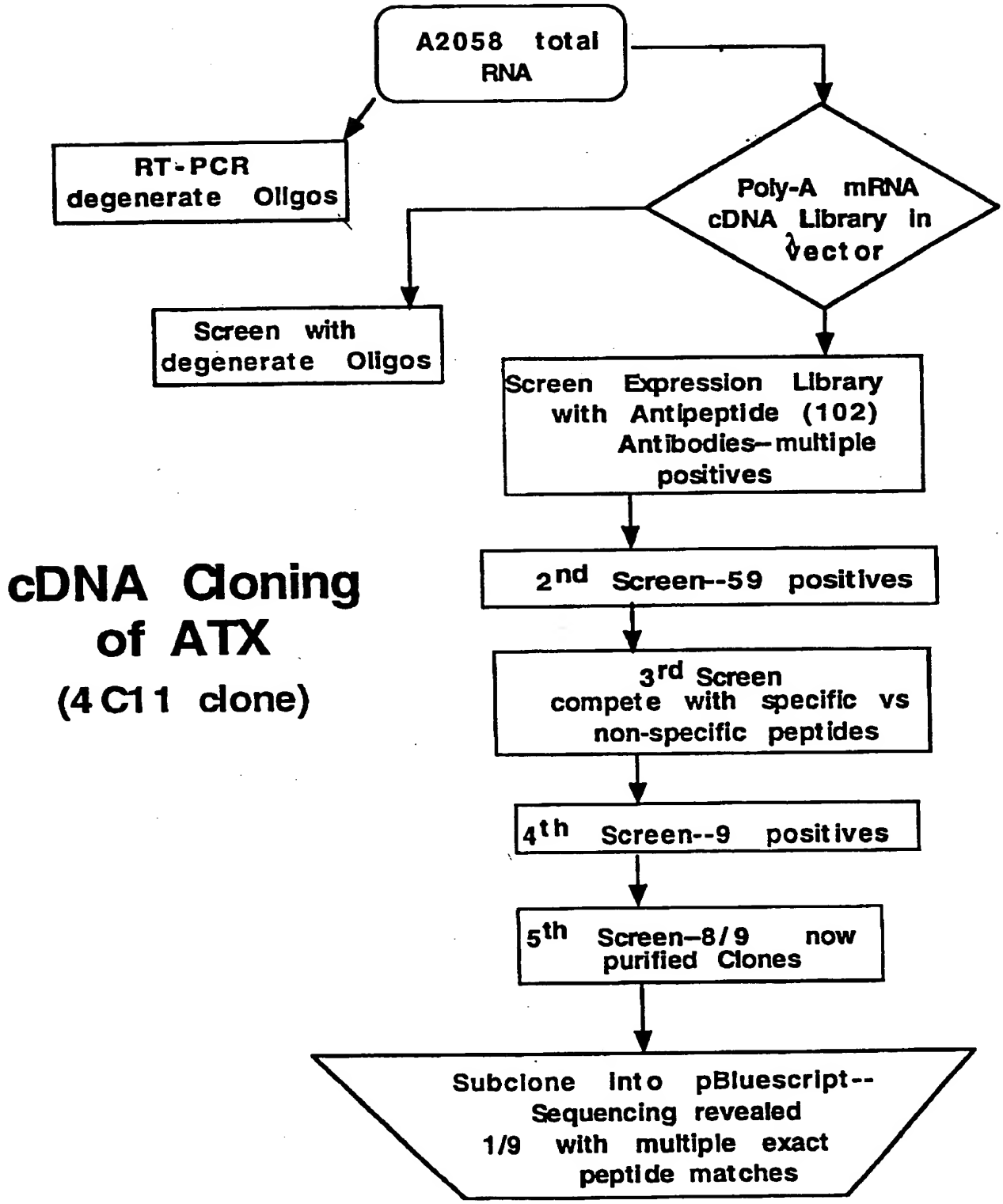

A2058 total RNA
RT-PCR degenerate Oligos
Poly-A mRNA cDNA Library in λ vector
Screen with degenerate Oligos
Screen Expression Library with Antipeptide (102) Antibodies--multiple positives
2nd Screen--59 positives
3rd Screen compete with specific vs non-specific peptides
4th Screen--9 positives
5th Screen--8/9 now purified Clones
Subclone into pBluescript--Sequencing revealed 1/9 with multiple exact peptide matches
cDNA Cloning of ATX
(4C11 clone)

FIGURE 12

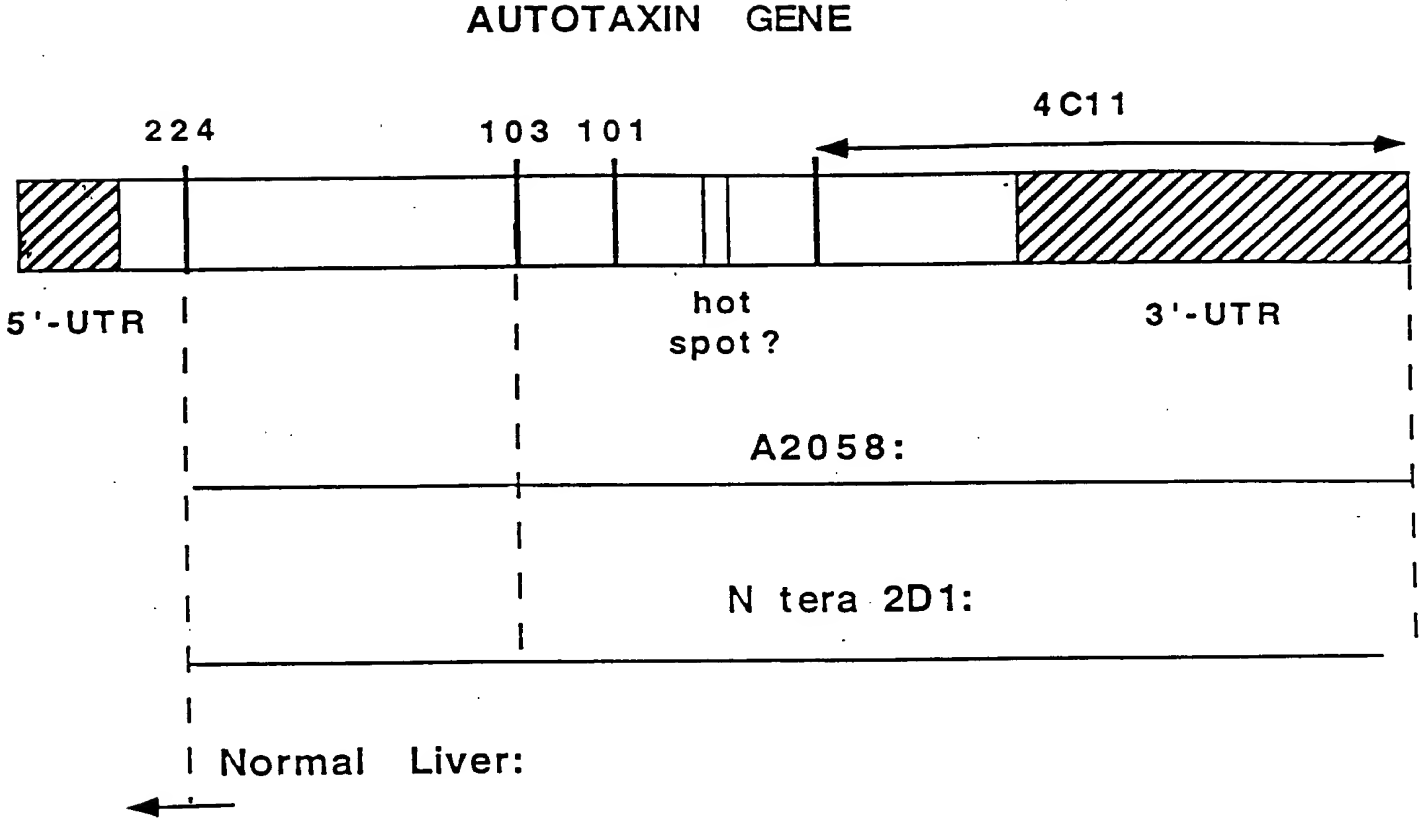

AUTOTAXIN GENE
224
103 101
4C11
5'-UTR
hot
spot?
3'-UTR
A2058:
N tera 2D1:
Normal Liver:

FIGURE 13

Match-up of ATX peptides with putative A2058 protein sequence

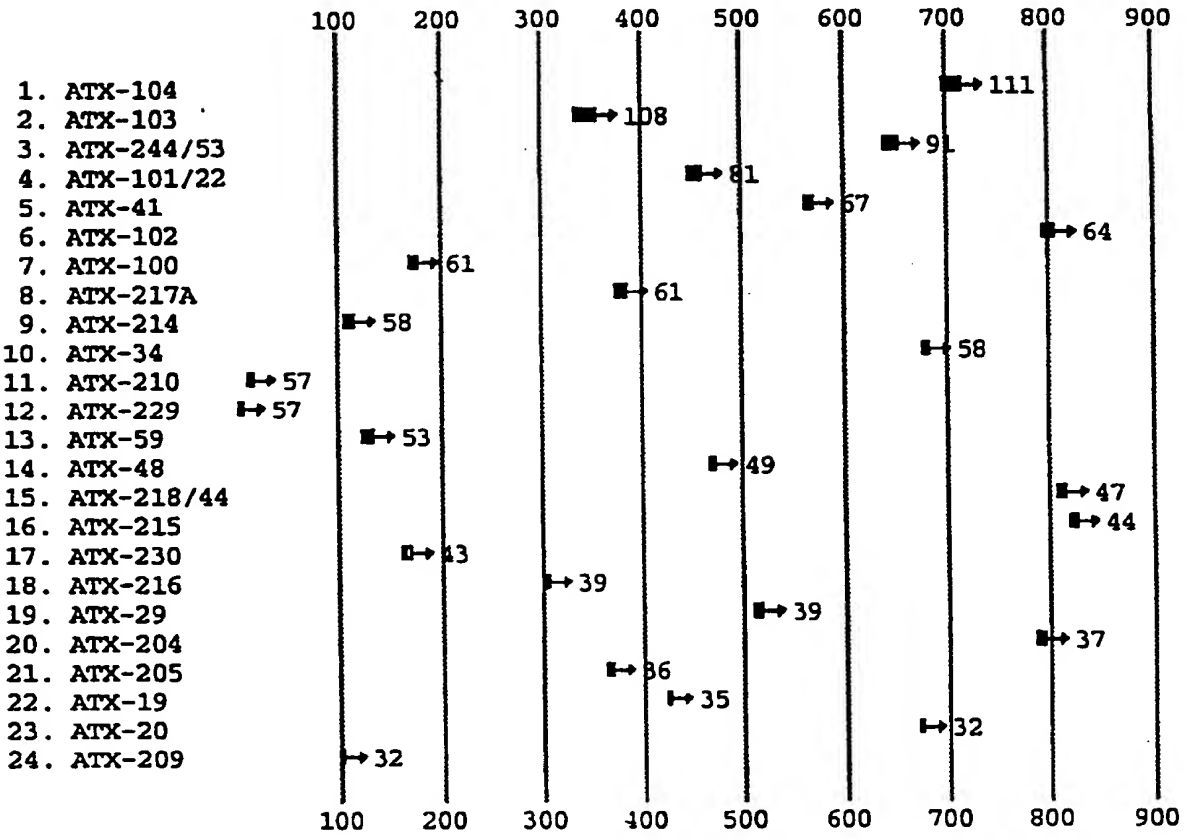


FIGURE 14

Match-up of ATX peptides with putative N-tera 2D1 protein sequence

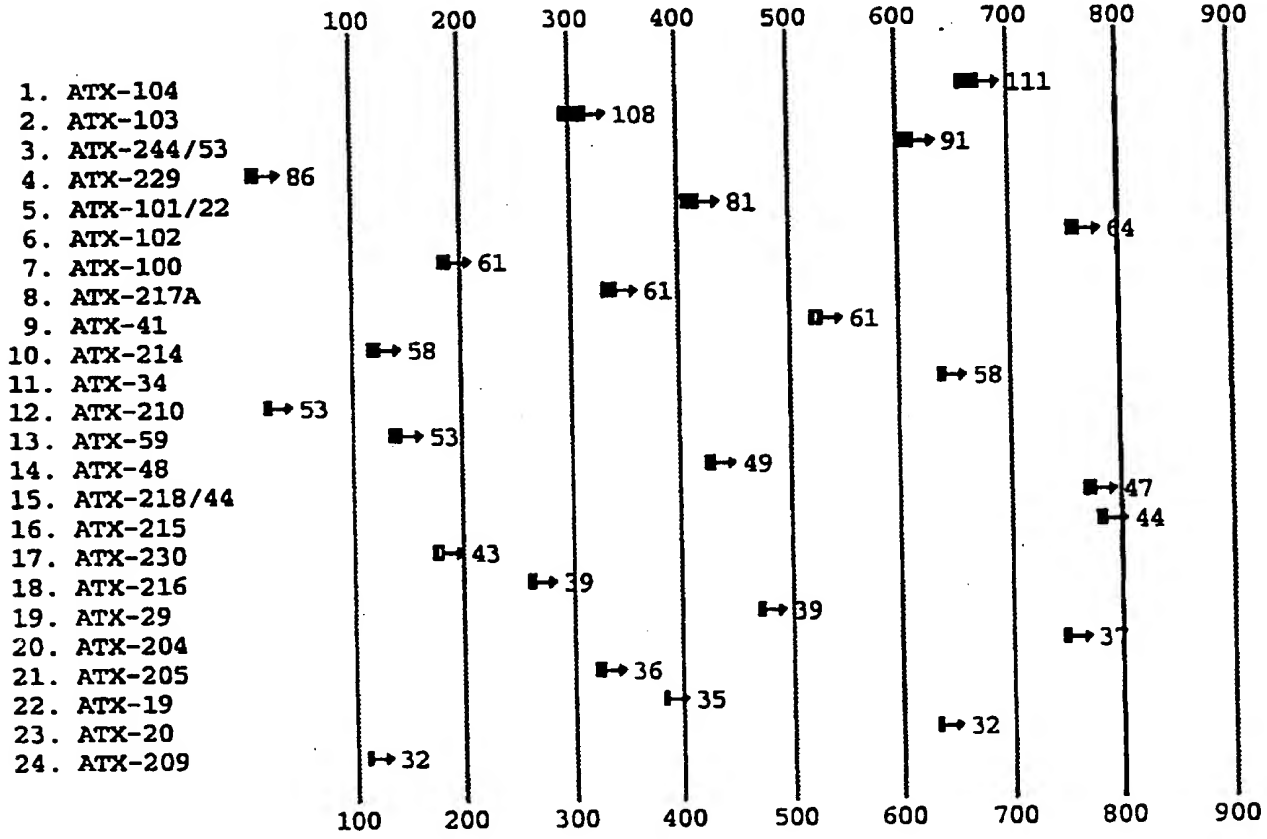
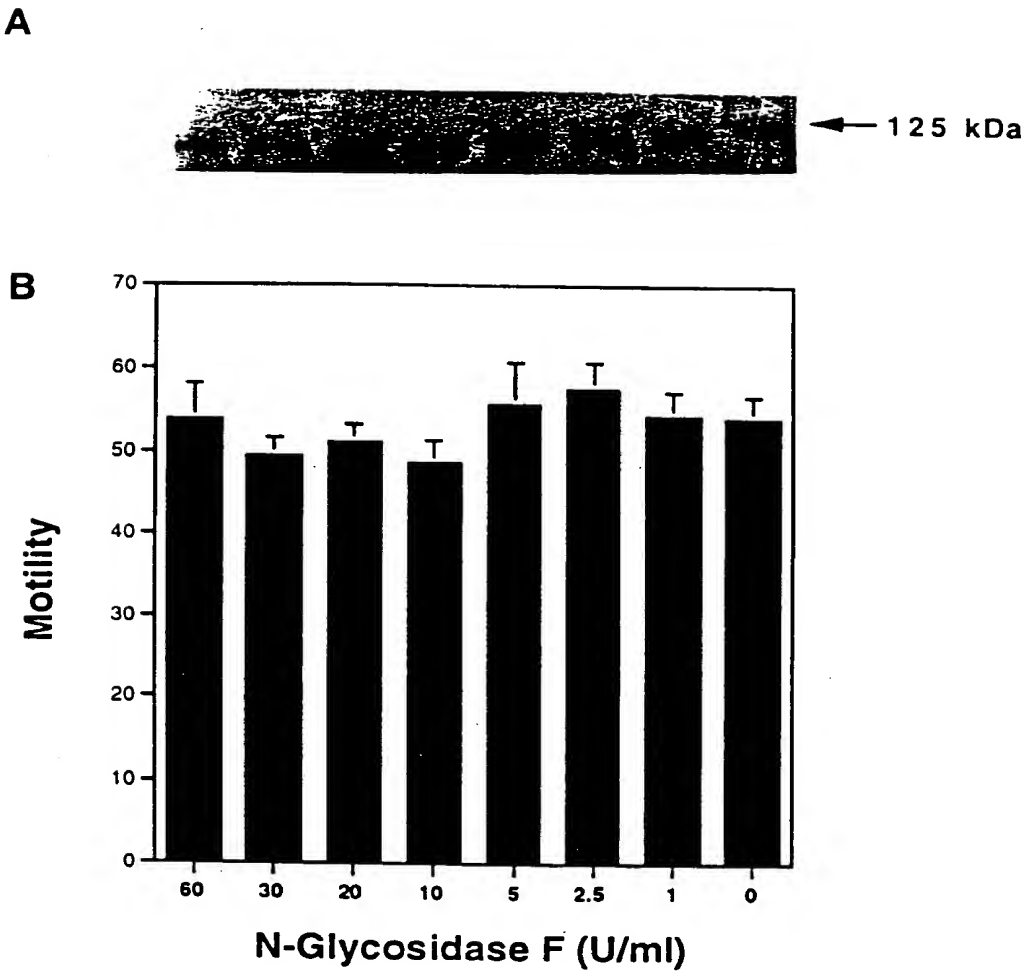


FIGURE 15

FIG. 16

FIG. 17


A
125 kDa
B
Motility
70
60
50
40
30
20
10
0
60
30
20
10
5
2.5
1
0
N-Glycosidase F (U/ml)

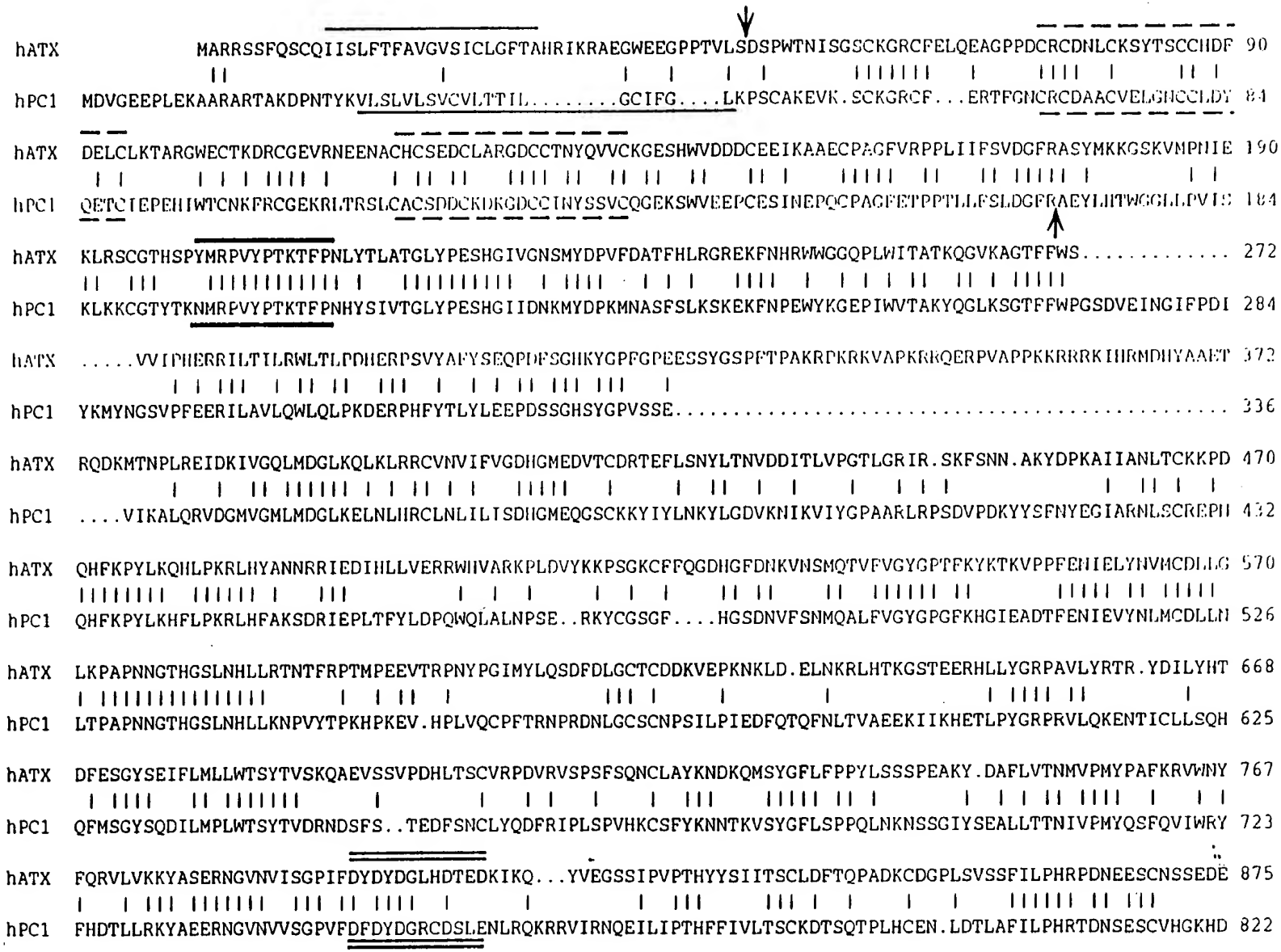
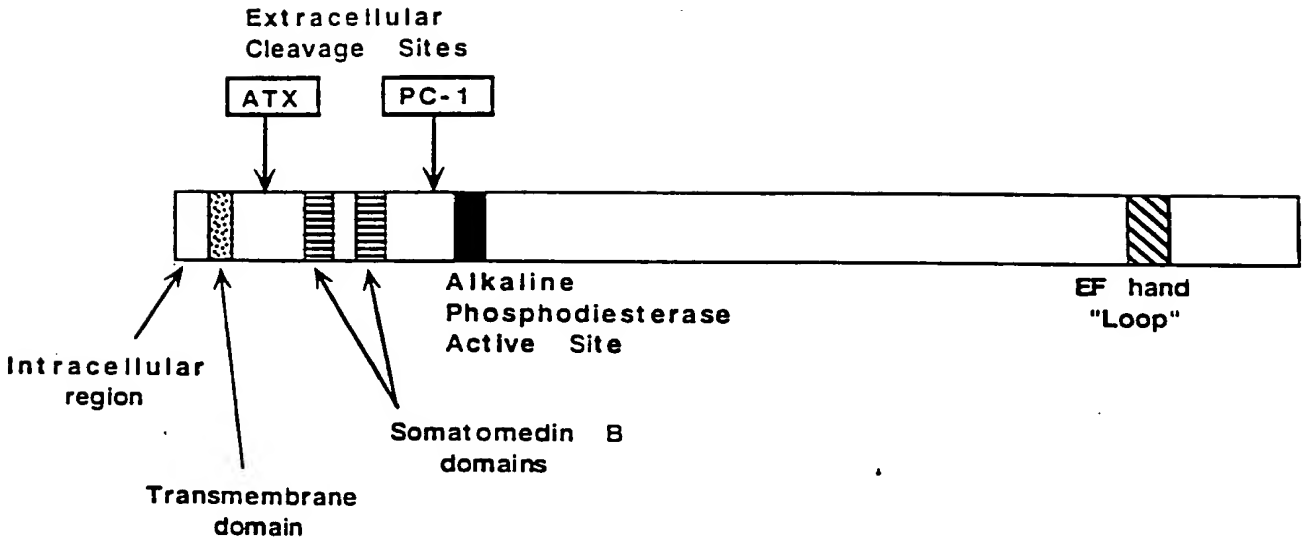

hATX MARRSSFQSCQIISLFTFAVGVSICLGFTAHRIKRAEGWEEGPPTVLSDSPWTNISGSCKGRCFELQEAGPPDCRCDNLCKSYTSCCHDF 90
hPC1 MDVGEEPLEKAARARTAKDPNTYKVLSLVLSVCVLTTIL.....GCIFG....LKPSCAKEVK.SCKGRCF...ERTFGNCRCDAACVELGHCCLDY 84
hATX DELCLKTARGWECTKDRCGEVRNEENACHCSEDCLARGDCCTNYQVVCKGESHWVDDDCEEIKAAECPAGFVRPPLIIFSVDGFRASYMKKGSKVMPNIE 190
hPC1 QETCIEPEHIWTCNKFRCGEKRLTRSLCACSDDCKDKGDCCINYSSVCQGEKSWVEEPCESINEPQCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVIS 184
hATX KLRSCGTHSPYMRPVYPTKTFPNLYTLATGLYPESHGIVGNSMYDPVFDATFHLRGREKFNHRWWGGQPLWITATKQGVKAGTFFWS..... 272
hPC1 KLKKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDI 284
hATX .....VVIPHERRILTILRWLTLPDHERPSVYAFYSEQPDFSGHKYGPFGPEESSYGSPFTPAKRDKRKVAPKRKQERPVAPPKKRRRKIHRMDHYAAET 372
hPC1 YKMYNGSVPFEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSE..... 336
hATX RQDKMTNPLREIDKIVGQLMDGLKQLKLRRCVNVIFVGDHGMEDVTCDRTEFLSNYLTNVDDITLVPGTLGRIR.SKFSNN.AKYDPKAIIANLTCKKPD 470
hPC1 ....VIKALQRVDGMVGMLMDGLKELNLHRCLNLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPN 432
hATX QHFKPYLKQHLPKRLHYANNRRIEDIHLLVERRWHVARKPLDVYKKPSGKCFFQGDHGFDNKVNSMQTVFVGYGPTFKYKTKVPPFENIELYNVMCDLLG 570
hPC1 QHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPQWQLALNPSE..RKYCGSGF....HGSDNVFSNMQALFVGYGPGFKHGIEADTFENIEVYNLMCDLLN 526
hATX LKPAPNNGTHGSLNHLLRTNTFRPTMPEEVTRPNYPGIMYLQSDFDLGCTCDDKVEPKNKLD.ELNKRLHTKGSTEERHLLYGRPAVLYRTR.YDILYHT 668
hPC1 LTPAPNNGTHGSLNHLLKNPVYTPKHPKEV.HPLVQCPFTRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQH 625
hATX DFESGYSEIFLMLLWTSYTVSKQAEVSSVPDHLTSCVRPDVRVSPSFSQNCLAYKNDKQMSYGFLFPPYLSSSPEAKY.DAFLVTNMVPMYPAFKRVWNY 767
hPC1 QFMSGYSQDILMPLWTSYTVDRNDSFS..TEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEALLTTNIVPMYQSFQVIWRY 723
hATX FQRVLVKKYASERNGVNVISGPIFDYDYDGLHDTEDKIKQ...YVEGSSIPVPTHYYSIITSCLDFTQPADKCDGPLSVSSFILPHRPDNEESCNSSEDE 875
hPC1 FHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCDSLENLRQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCEN.LDTLAFILPHRTDNSESCVHGKHD 822
hATX SKWVEELMKMHTARVRDIEHLTSLDFFRKTSRSYPEILTLKTYLHTYESEI 915
hPC1 SSWVEELLMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQED 873

FIG. 18

FIG. 19


Extracellular
Cleavage Sites
ATX
PC-1
Alkaline
Phosphodiesterase
Active Site
EF hand
"Loop"
Intracellular
region
Somatomedin B
domains
Transmembrane
domain